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by William N. Shaw

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# Seasonal Gonadal Cycle of the Male Soft Shell Clam, Mya arenaria, in Maryland

By

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## ABSTRACT

Seasonal changes in the gonad of the male soft-shell clam, Mya arenaria, were observed histologically in samples collected from May 1961 to June 1963 in the Tred Avon River, located on the eastern shore of Chesapeake Bay, Md. In the summer no active spermatogenesis was found. The aveoli contained many atypical cell inclusions with 1 to 16 nuclei. In about 50 percent of the male clams, the aveoli also contained sperm-balls, a grouping of unspawned sperm.

Spermatogenesis started in August. Spawning began about mid-October and was completed early in November 1961, while in 1962 spawning was first observed about mid-September and was completed about mid-October.

A second cycle of spermatogenesis followed shortly after the completion of fall spawning. In April many clams were entering the inactive stage and spermatogenesis was not completed. In others, spermatozoa occupied the center of each alveolus. These sperm were surrounded by follicle cells containing inclusions. No spring spawning was observed in clams in the Tred Avon River; instead, those sperm present were grouped into balls and held until fall.

No hermaphrodites were found, and possible sex change from male to female seems doubtful.

## INTRODUCTION

An understanding of the seasonal spermatogenic cycle of the soft-shell clam, Mya arenaria, in Chesapeake Bay is essential before the fishery for this commercially important species can be properly managed. Coe and Turner (1938) described the development of the gonad in M. arenaria at New Haven, Conn., and stated that there was one reproductive cycle each year. Ropes and Stickney (personal communication) found a single annual spawning cycle for the soft-shell clam in the Gulf of Maine. Further south in Wickford Harbor, Narragansett Bay, R.I., Landers (1954) found Mya larvae from May to mid-July and a smaller second wave from mid-August to November. A lack of larvae between the two waves indicated that spawning occurred biannually in that region. Rogers (1959), working at Solomons Island on the western shore of Chesapeake Bay, reported that the soft-shell clam spawned in the fall, and he believed a second period of spawning was thought to have occurred in the spring. Pfitzenmeyer (1962) showed from clam larvae counts and set traps that the Maryland soft-shell clam

in the Patuxent River spawned twice a year; he mentioned only briefly his findings from histological examination of Mya collected in the area. From histological examination Shaw (1964) demonstrated that there were two reproductive cycles each year in the female soft-shell clam on the eastern shore of Chesapeake Bay, but the second or spring cycle was not completed. Apparently Mya in Maryland waters, with two seasonal reproductive cycles, must differ from its more northern relative in Maine and Long Island Sound, Conn., which has only one spawning cycle each year.

The purpose of this paper is to describe the seasonal gonadal cycle of the male M. arenaria observed in histological sections from samples collected from May 1961 to June 1963 in the Tred Avon River, Md. Information collected on the seasonal occurrence of Mya larvae, seasonal setting records in bottle collectors, and results from histological examination of M. arenaria from other areas of Chesapeake Bay are also given.

NOTE: A paper read July 24, 1963, Annual Meeting of the National Shellfisheries Association, Washington, D.C.

## MATERIALS AND METHODS

The clams were collected from shallow water in the Tred Avon River opposite the Bureau of Commercial Fisheries Biological Laboratory in Oxford, Md. These samples were taken weekly during periods of active spermatogenesis and monthly during periods of maturation inactivity. In spring 1963, additional samples were collected from the Potomac, Patuxent, and Chester Rivers.

Each sample, consisting of 10 or more clams 2 or more inches long, was fixed in Kahle's fluid (Guyer, 1953), dehydrated in alcohol, cleared in xylene, and mounted in paraffin. The gonad of each clam was then sectioned at about 7 microns with a standard rotary microtome. The sections were stained in either Harris' or Delafield's hematoxylin and counterstained with eosin. Of the 1,063 clams examined about 50 percent were males.

## RESULTS

Spermatogenesis was inactive in male clams collected from May through July 1961 and 1962. Each alveolus consisted of follicular cells with many atypical cell inclusions with 1 to 16 nuclei (fig. 1A). In addition to these multinucleated inclusions, groups of sperm, or sperm-balls, were found in about 50 percent of the males examined (fig. 1B). Probably these sperm-balls were derived from sperm that were not released from the previous spawning period. Coe and Turner (1938) noted bodies similar in size but described them as nuclei that were pycnotic. Loosanoff (1937) reported that spermatozoa remaining in Mercenaria (Venus) mercenaria underwent cytolysis, but in Mya this was not observed.

In both 1961 and 1962, active spermatogenesis began in August. Primary and secondary spermatogonia were observed along the base of the alveolar walls, with secondary spermatogonia protruding toward the center of the alveoli. By early September spermatogenesis was progressing at a rapid rate. The more advanced sex cells, primary and secondary spermatocytes, and spermatids were closer to the center of the alveolus (fig. 2). During this period all stages of spermatogenesis could be found.

In 1961, ripe clams were found in the latter part of September, while in 1962 this stage was observed several weeks earlier. Just prior to spawning, each alveolus consisted of mature spermatozoa, with their tails filling the center of the lumen (fig. 3). Only a few of the inclusions so prevalent during the summer were present. The sperm-balls, numerous earlier, had disappeared, indicating that the sperm had been released into the center of the lumen.

Spawning was first observed on October 12, 1961, and September 17, 1962. In partially

spawned-out clams (fig. 4) the rows of sperm were more separated, and the lumen was not as fully packed with sperm tails. A few inclusions could still be found near the alveolar walls. In 1961 and 1962, spawning was completed by early November. Each alveolus contained follicular cells with a few inclusions, far less than found during the summer (fig. 5A). In addition, many alveoli also contained unspawned sperm (fig. 5B). Instead of the sperm undergoing cytolysis, they were grouped into sperm-balls and retained throughout the winter (fig. 6).

A second cycle of spermatogenesis began shortly after fall spawning (fig. 7). Primary and secondary spermatogonia were developing along the base of the alveolar walls. From January through March little further development was observed. The follicular cells had only a few multinucleated inclusions. Also, the gonads appeared watery.

During April, when active spermatogenesis was expected with a rise in temperature, the spermatogonia apparently underwent cytolysis, and the clam entered the summer or inactive stage (fig. 1A). In many clams it appeared that sperm were liberated from the sperm-balls into the center of the lumen. Coe and Turner (1938) reported that an aberrant mode of meiosis occurred in Mya and the multinucleated cells (inclusions) form spermatids that later transform into spermatozoa. Atypical spermatogenesis was not observed in Maryland clams.

In spring 1962, the clams had no primary and secondary spermatocytes or spermatids; during spring 1963, only 10 percent of the clams contained these stages of spermatogenesis. In no microscopic sections were the clams as ripe as during the previous fall. Instead, the sperm present were surrounded by follicular cells with inclusions (fig. 8). Spring spawning was not observed either in 1962 or 1963. As in the fall, the unspawned sperm were grouped into balls and carried in the follicular cells throughout the summer (fig. 1B).

In addition to the histological examination of the seasonal changes in the gonad of the soft-shell clam, plankton samples were collected and studied for the occurrence of Mya larvae, and the contents of bottle collectors (after Thorson, 1946) were examined for newly settled Mya. In fall 1961 and 1962 Mya larvae and set were found in the Tred Avon River. No larvae or set were observed in spring 1962, and no larvae were found in spring 1963. Five newly set Mya were caught in two bottles exposed from May 6 to June 17, 1963. The presence of these juvenile clams indicated that some spawning had occurred during spring 1963, although my examination of the testis and the plankton indicated no apparent spawning had taken place. It is possible that these clams developed from eggs spawned outside the Tred Avon River. Pfitzenmeyer (1962) observed



spring spawning of the soft-shell clam for the years 1957, 1958, and 1959 at Solomons, Md., but, except for the five Mya caught in the bottle collector, I had no evidence of spring spawning in the Tred Avon River either in 1962 or 1963.

In the spring of 1963, to compare the condition of Tred Avon River clams with clams of other areas, I made collections from a salt-water tank at the Chesapeake Biological Laboratory, Solomons, Md. (April 16), and in the Patuxent River (April 25 and May 22), Potomac River (May 22), and Chester River (May 22). All samples from the Patuxent, Potomac, and Chester Rivers were in a similar gonadal stage as those collected from our local waters at the corresponding time. None showed signs of spawning or preparing to spawn. The clams from Chesapeake Biological Laboratory were in all stages of gametogenesis. Many were approaching ripeness, several were ripe, while others were entering the summer or inactive stage. These differences in gonadal development could be caused by the unnatural conditions existing in the salt-water tanks. Unfortunately, the source of these clams and how long they had been in the tanks were not known.

No hermaphrodites were found in more than 800 clams. Coe and Turner (1938) found only 3 hermaphrodites in 1,000 individuals. The possibility of sex change from male to female in the soft-shell clam seems doubtful. Except in instances where the clams were in very poor condition, the sex was never in doubt. Each sex is easily recognizable throughout the year by their inclusions (only multinucleated in males) and sex cells. The indifferent stage, found in oysters (Loosanoff, 1942), was not present in Mya.

## CONCLUSIONS

Histological examination of the male soft-shell clam from the Tred Avon River revealed, as in the female, two cycles of gonadal development each year, one that begins in August, and a second initiated shortly after fall spawning is completed. Evidence that spring setting of limited nature can take place in the Tred Avon River was observed when five Mya were found in our bottle collectors. Where this spawning took place is not known, since the clams collected in front of the laboratory showed no signs of spawning.

The formation of sperm-balls derived from the unspent sperm in Mya has not been found in Mercenaria (Venus) mercenaria (Loosanoff, 1937) or Crassostrea virginica (Loosanoff, 1942). In these two species the unspawned sperm undergo cytolysis. The sperm-balls in Mya were found after fall spawning and in summer. Present evidence indicates that the

major source of sperm found in the gonad during the spring is derived from these balls, while in the fall sperm is derived both from normal spermatogenesis and from sperm-balls.

Future ecological research may reveal the limiting factors in spring spawning. In order to interpret age, growth, mortality, and survival data, it will be necessary to watch closely the spawning cycle of Mya each year in the Chesapeake Bay area.

## ACKNOWLEDGMENTS

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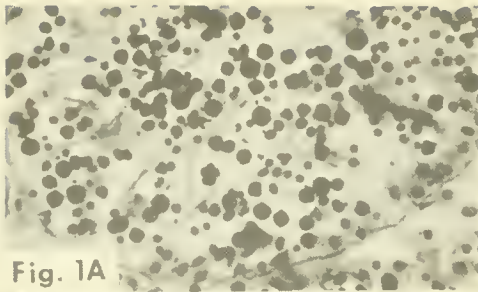


Fig. 1A

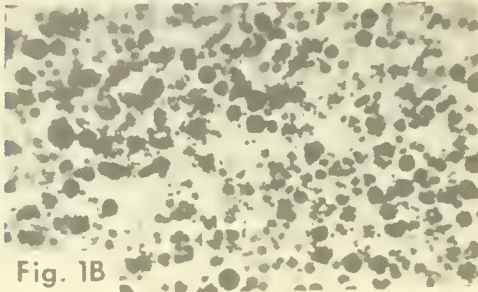


Fig. 1B

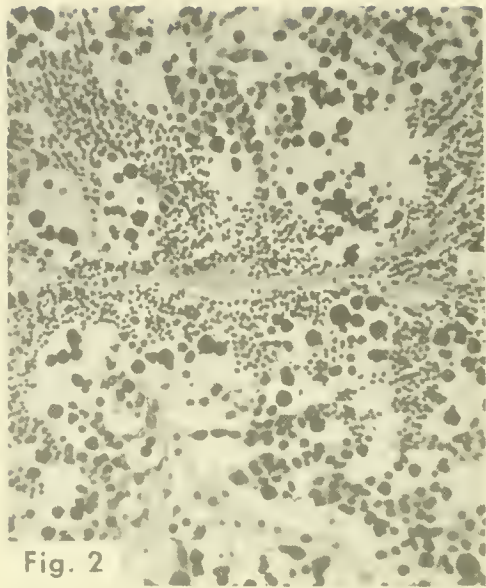


Fig. 2

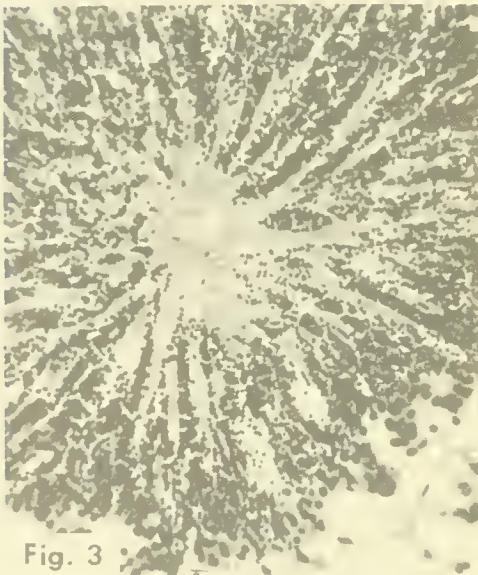


Fig. 3

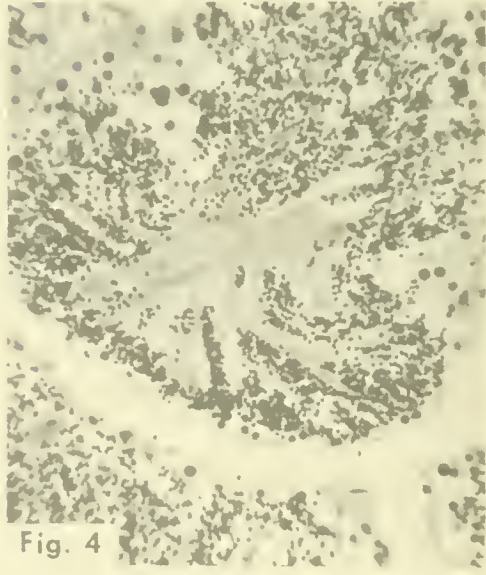


Fig. 4

Figure 1A.--Summer, or inactive, stage. Follicular cells with multinucleated bodies called inclusions, male, August, X430.

Figure 1B.--Summer, or inactive, stage. Follicular cells with inclusions and sperm balls, male, August, X430.

Figure 2.--Clam partially ripe, September. X430.

Figure 3.--Ripe male clam, October. X430.

Figure 4.--Partially spawned out, October, X430.



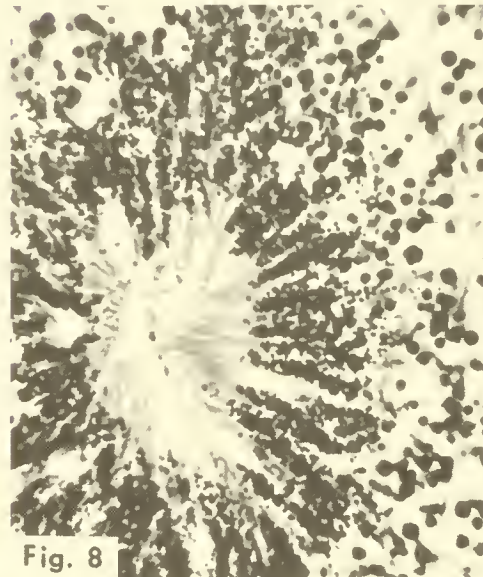
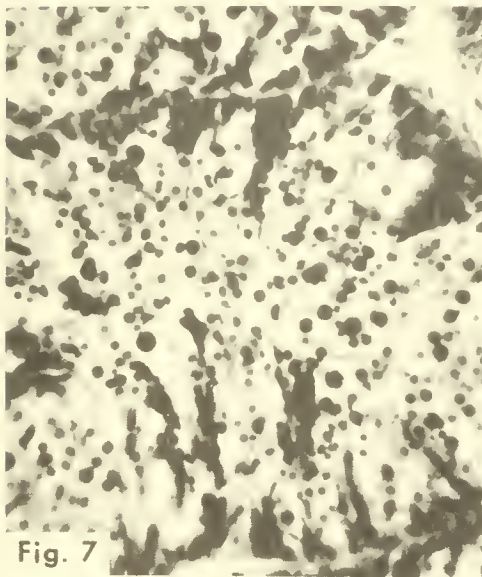
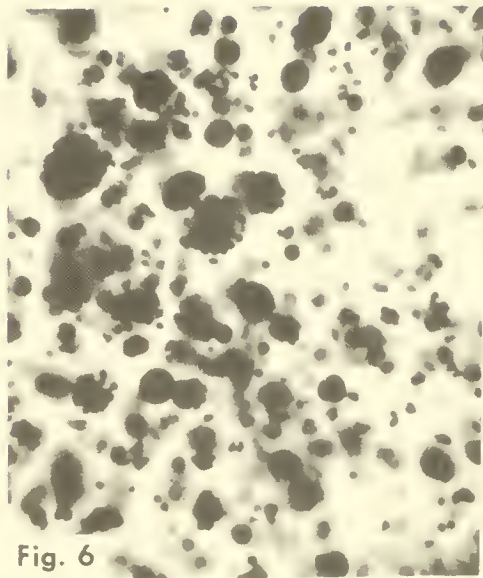
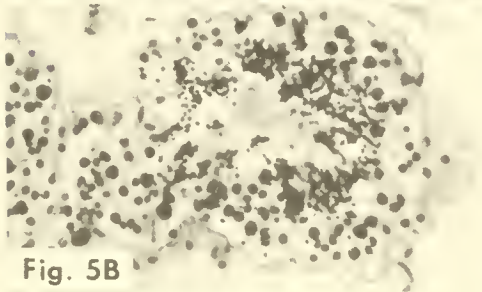
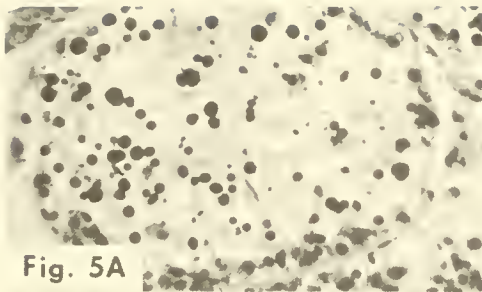


Figure 5A.--Spawned out, November, X430.

Figure 5B.--Spawned out, with some sperm retained, November, X430.

Figure 6.--Winter clam with sperm-balls, January, X970.

Figure 7.--Early spermatogenesis of second cycle, January, X430.

Figure 8.--Partially ripe clam. Notice the inclusions between sperm and alveolar wall, April, X430.

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